DEPARTMENT OF HEALTH AND HUMAN SERVICES

Quality Control – Introduction

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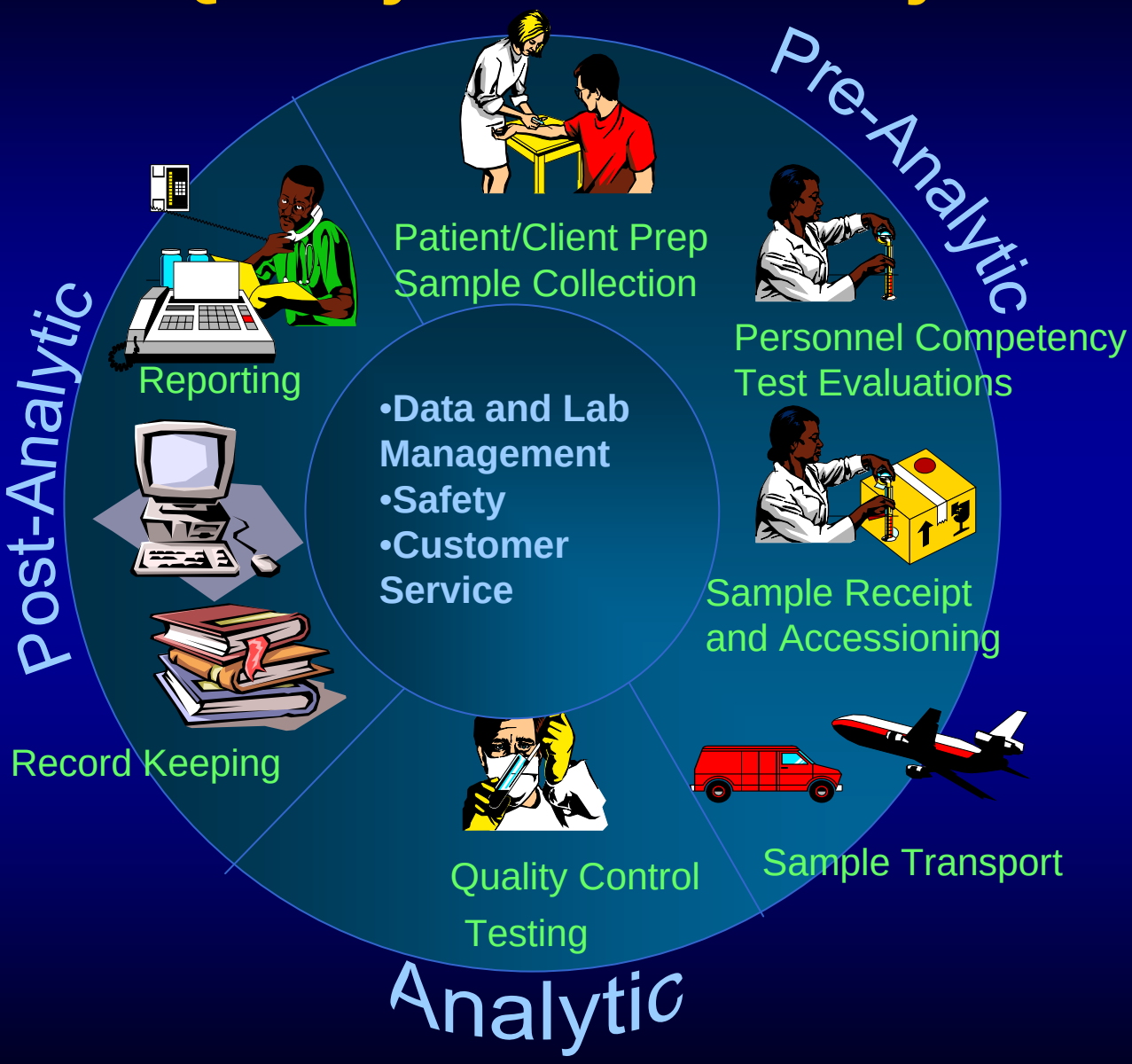
The Quality System

The Quality Assurance Cycle



Quality Control

- Definitions
- Qualitative Quality Control
- Quantitative QC – How to implement
  - Selection and managing control materials
  - Analysis of QC data
  - Monitoring quality control data

What is Quality Control?

- Process or system for monitoring the quality of laboratory testing, and the accuracy and precision of results
- Routinely collect and analyze data from every test run or procedure
- Allows for immediate corrective action

Designing a QC Program –

- Establish written policies and procedures
  - Corrective action procedures
- Train all staff
- Design forms
- Assure complete documentation and review

Qualitative vs.Quantitative

- Quantitative test
  - measures the amount of a substance present
- Qualitative test
  - determines whether the substance being tested for is present or absent

Qualitative QC

- Quality control is performed for both, system is somewhat different
- Controls available
  - Blood Bank/Serology/Micro
  - RPR/TPHA
  - Dipstick technology
  - Pregnancy

Stains, Reagents, Antisera

- Label containers
  - contents
  - concentration
  - date prepared
  - placed in service
  - expiration date/shelf life
  - preparer

Media Preparation

- Record amount prepared
- Source
- Lot number
- Sterilization method
- Preparation date
- Preparer
- pH
- Expiration date

Microbiology QC

- Check:
  - Sterility
  - Ability to support growth
  - Selective or inhibitory characteristics of the medium
  - Biochemical response
- Frequency
  - Test QC organisms with each new batch or lot number
- Check for growth of fastidious organisms on media of choice – incubate at time and temp recommended
- RECORD Results on Media QC form

Quality Control: Stains and Reagents

- Gram stain QC
  - Use gram positive and gram negative organisms to check stain daily
- Other :
  - Check as used – positive and negative reactions

Stock QC organisms

- Organisms to be maintained must be adequate to check all media and test systems.
  - *E. coli* – MacConkey, EMB, susceptibility tests
  - *Staphylococcus aureus* – Blood agar, Mannitol Salt, susceptibility tests
  - *Neisseria gonorrhoeae* – chocolate, Martin-Lewis

Detecting Errors

- Many organisms have predictable antimicrobial test results
  - *Staphylococcus* spp. are usually susceptible to vancomycin
  - *Streptococcus pyogenes* are always susceptible to penicillin
  - *Klebsiella pneumoniae* are resistant to ampicillin

Sources of Error

- If you encounter an unusual pattern
  - rule out error by checking identification of organisms
  - repeat antimicrobial susceptibility test

- Report if repeat testing yields same result, or refer the isolate to a reference laboratory for confirmation

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Quality Control – Quantitative Tests

How to implement a laboratory quality control program

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Implementing a QC Program – Quantitative Tests

- Select high quality controls
- Collect *at least* 20 control values over a period of 20-30
  days for each level of control
- Perform statistical analysis
- Develop Levey-Jennings chart
- Monitor control values using the Levey-Jennings chart and/or Westgard rules
- Take immediate corrective action, if needed
  - Record actions taken

Selecting Control Materials
Calibrators

- Has a known concentration of the substance (analyte) being measured
- Used to adjust instrument, kit, test system in order to standardize the assay
- Sometimes called a standard, although usually not a true standard
- This is *not* a control

Selecting Control Materials
Controls

- Known concentration of the analyte
  - Use 2 or three levels of controls
  - Include with patient samples when performing a test
- Used to validate reliability of the test system

Control Materials
Important Characteristics

- Values cover medical decision points
- Similar to the test specimen (matrix)
- Available in large quantity
- Stored in small aliquots
- Ideally, should last for at least 1 year
- Often use biological material, consider bio-hazardous

Managing Control Materials

- Sufficient material from same lot number or serum pool for one year's testing
- May be frozen, freeze-dried, or chemically preserved
- Requires very accurate reconstitution if this step is necessary
- Always store as recommended by manufacturer

Sources of QC Samples

- Appropriate diagnostic sample
- Obtained from:
  - Another laboratory
  - EQA provider
- Commercial product

Types of Control Materials

- Assayed
  - mean calculated by the manufacturer
  - must verify in the laboratory
- Unassayed
  - less expensive
  - must perform data analysis
- “Homemade” or “In-house”
  - pooled sera collected in the laboratory
  - characterized
  - preserved in small quantities for daily use

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Preparing In-House Controls

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Criteria for Developing Quality Controls for HIV

- Low positive
- Between the cut off and positive control
- At a level where variability can be followed
- Generally ~2 times the cut off

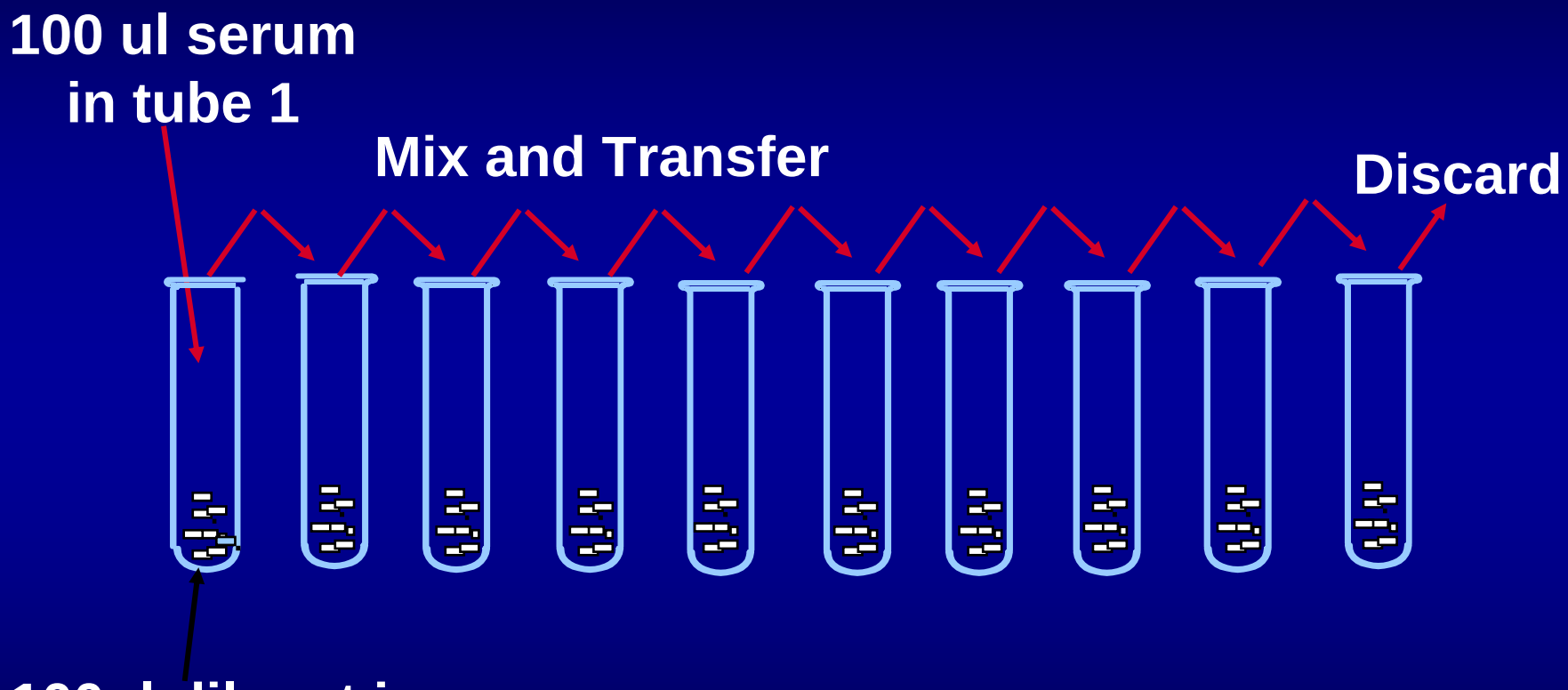
Production of a QC Sample - Production Protocol

- Materials
- Calculation of Volume
  - stock sample
    - diluent
    - QC batch
- Method
- Validation Acceptance Criteria
  - batch
  - stability

Process for Preparing In-house Controls

- Serial dilution of high positive stock sample
- Select suitable dilution
- Produce large batch
- Test stability
- Test batch variation
- Dispense, label, store

Making Suitable Dilutions

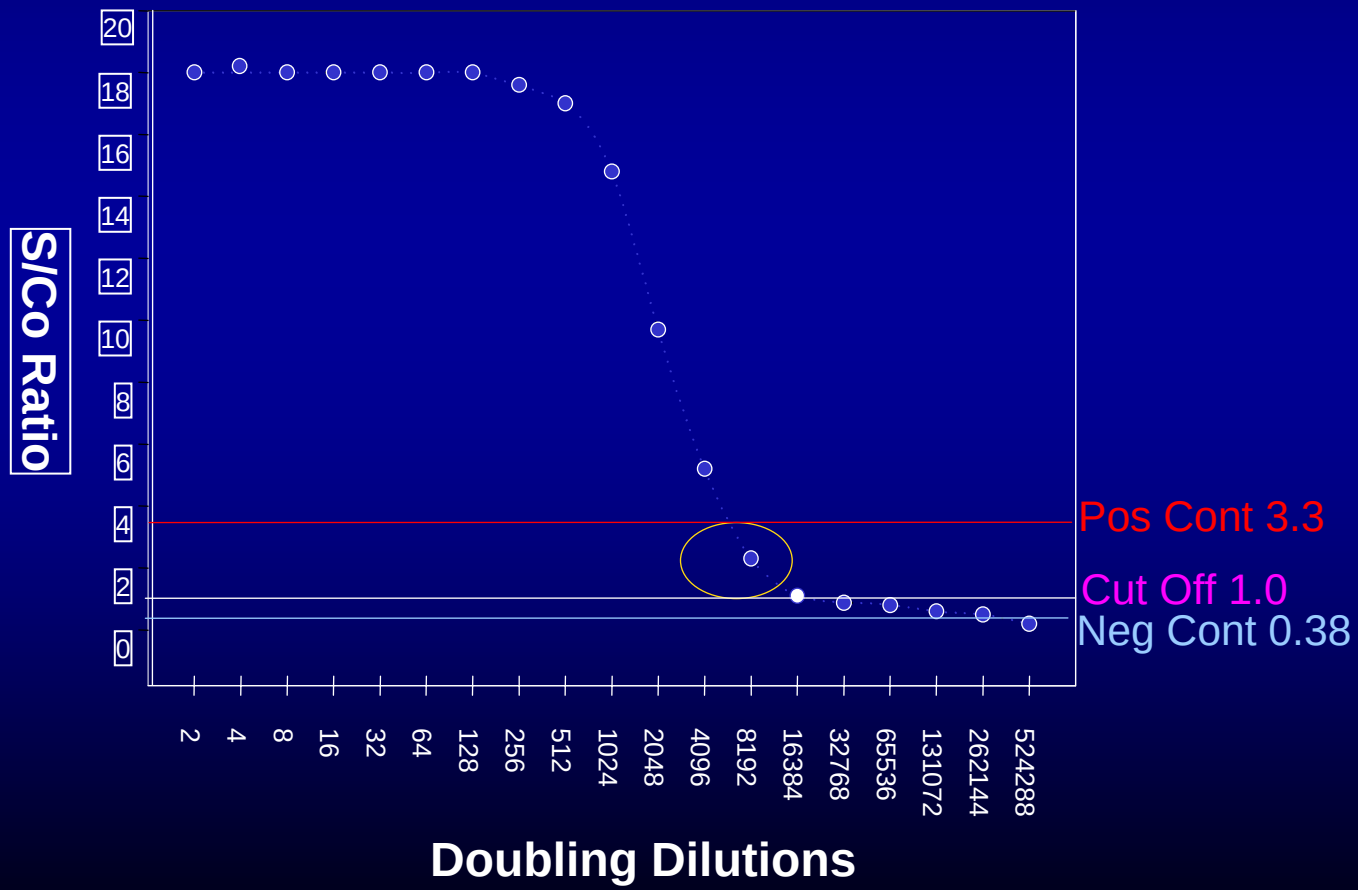


Each tube is a 1:2 dilution

of the previous tube

Selecting a Suitable Sample Dilution

Serial Dilutions on Abbott AxSYM HIV-1/HIV-2 MEIA



Batch Production

- Prepare positive sample
  - centrifuge
  - heat inactivate
- Mix positive sample in diluent
  - magnetic stirrer
- Bottle batch in numbered lots of suitable volume

Stability Testing

- Assess the rate of deterioration

QC Sample Storage	Day 7	Day 14	Day 21	Day 28
-20c	✓	✓	✓	✓
4c	✓	✓	✓	✓
16-25°C	✓	✓	✓	✓

Batch Validation

- Dispense aliquots
- Test aliquots
- Confirm desired titre level
  - compare against target value
- Confirm minimal batch variation
  - acceptable if CV <20%
  - aim for <10%

Storage of QC Samples

- Validated batch aliquoted into smaller ‘user friendly’ volumes for storage
- Establish a storage protocol:
  - store at -20^{o}C
  - in use vials stored at 4^{o}C
  - use 0.5 ml vial maximum of one week
  - freeze-dried
    (requires accurate reconstitution)
  - chemically preserved

Quality Control -Quantitative

Analysis of QC Data

How to carry out this analysis?

- Need tools for data management and analysis
  - Basic statistics skills
  - Manual methods
    - Graph paper
    - Calculator
  - Computer helpful
    - Spreadsheet
- Important skills for laboratory personnel

Analysis of Control Materials

- Need data set of at least 20 points, obtained over a 30 day period
- Calculate mean, standard deviation, coefficient of variation; determine target ranges
- Develop Levey-Jennings charts, plot results

Establishing Control Ranges

- Select appropriate controls
- Assay them repeatedly over time
  - at least 20 data points
- Make sure any procedural variation is represented:
  - different operators
  - different times of day
- Determine the degree of variability in the data to establish acceptable range

Measurement of Variability

- A certain amount of variability will naturally occur when a control is tested repeatedly.
- Variability is affected by operator technique, environmental conditions, and the performance characteristics of the assay method.
- The goal is to differentiate between variability due to chance from that due to error.

Measures of Central Tendency

- Data are frequently distributed about a central value or a central location
- There are several terms to describe that central location, or the ‘central tendency’ of a set of data

Measures of Central Tendency

- Median = the value at the center (midpoint) of the observations
- Mode = the value which occurs with the greatest frequency
- Mean = the calculated average of the values

Calculation of Mean

$$(\overline{X}) = \frac{X_1 + X_2 + X_3 \ldots + X_n}{n}$$

$\overline{X}$ = Mean

X_1 = First result

X_2 = Second result

X_n = Last result in series

n – Total number of results

Calculation of Mean: Outliers

1. 192 mg/dL
2. 194 mg/dL
3. 196 mg/dL
4. 196 mg/dL
5. 160 mg/dL
6. 196 mg/dL
7. 200 mg/dL
8. 200 mg/dL
9. 202 mg/dL
10. 255 mg/dL
11. 204 mg/dL
12. 208 mg/dL
13. 212 mg/dL

Calculation of Mean

1) 192 mg/dL
2) 194 mg/dL
3) 196 mg/dL
4) 196 mg/dL
5) 196 mg/dL
6) 200 mg/dL
7) 200 mg/dL
8) 202 mg/dL
9) 204 mg/dL
10) 208 mg/dL
11) 212 mg/dL

Sum = 2,200 mg/dL

- Mean = the calculated average of the values
- The sum of the values ($X_1 + X_2 + X_3 \ldots X_{11}$) divided by the number (n) of observations
- The mean of these 11 observations is (2200 ÷ 11) = 200 mg/dL

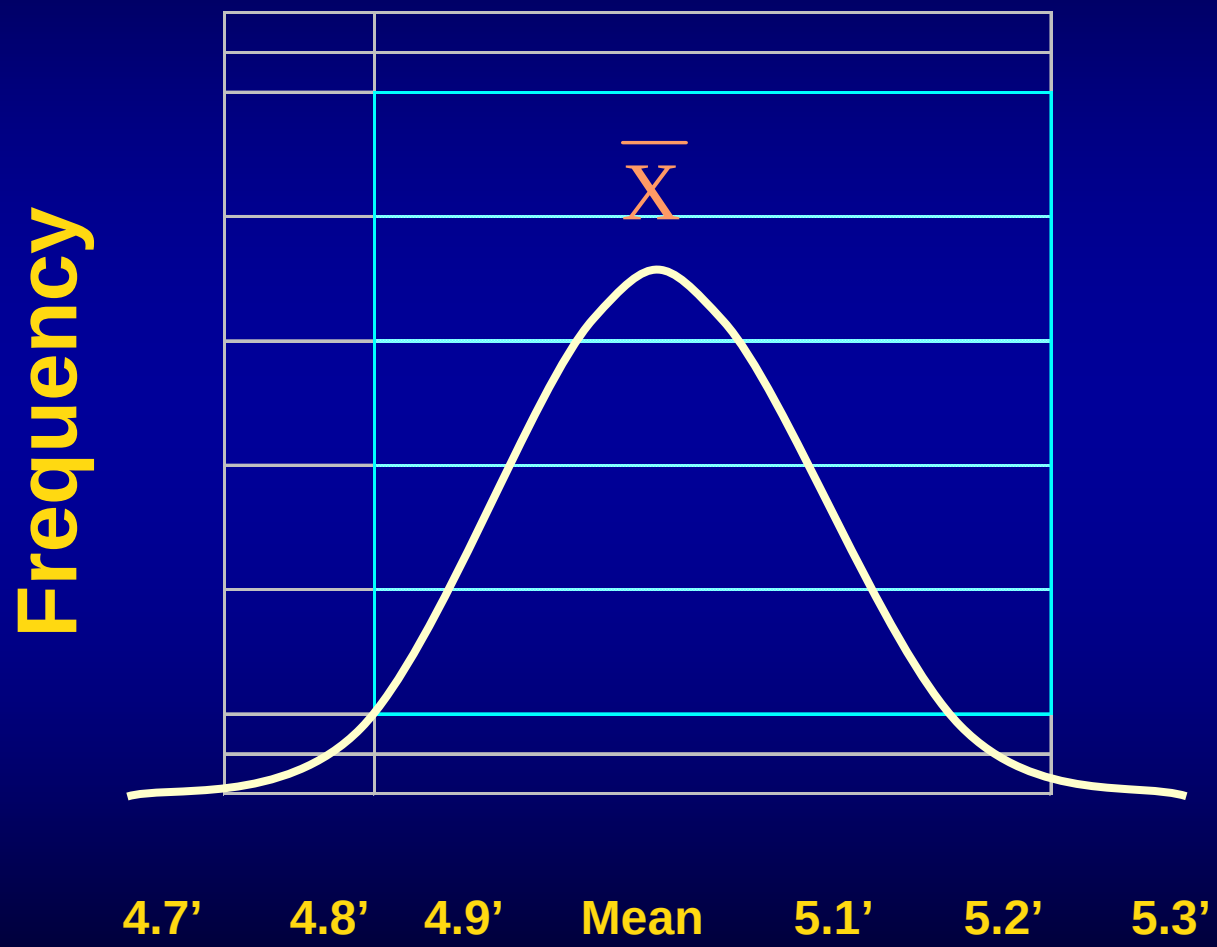
Calculation of Mean: ELISA Tests

- Collect optical density (OD) values for controls for each assay run
- Collect cutoff (CO) value for each run
- Calculate ratio of OD to CO (OD/CO) for each data point or observation
  - This ratio standardizes data
- Use these ratio values to calculate the mean

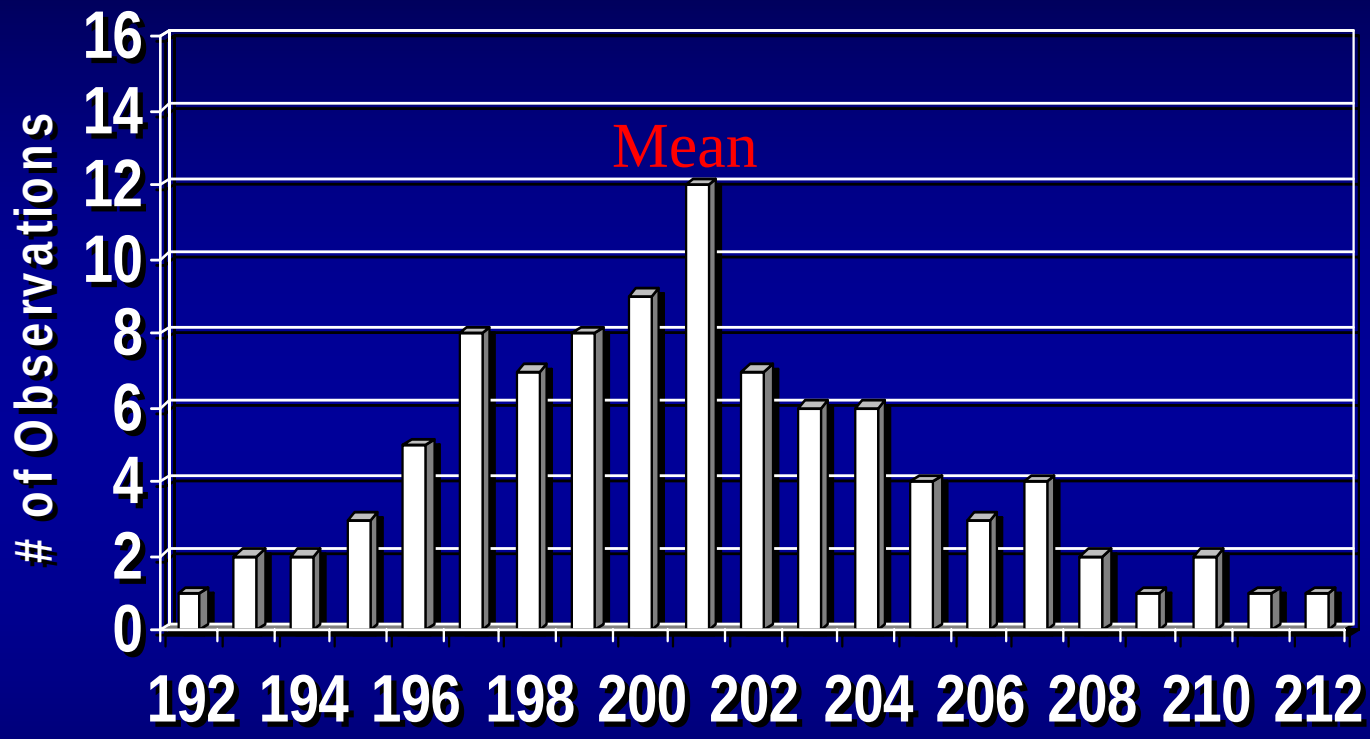
Normal Distribution

- All values are symmetrically distributed around the mean
- Characteristic “bell-shaped” curve
- Assumed for all quality control statistics

Normal Distribution



Normal Distribution

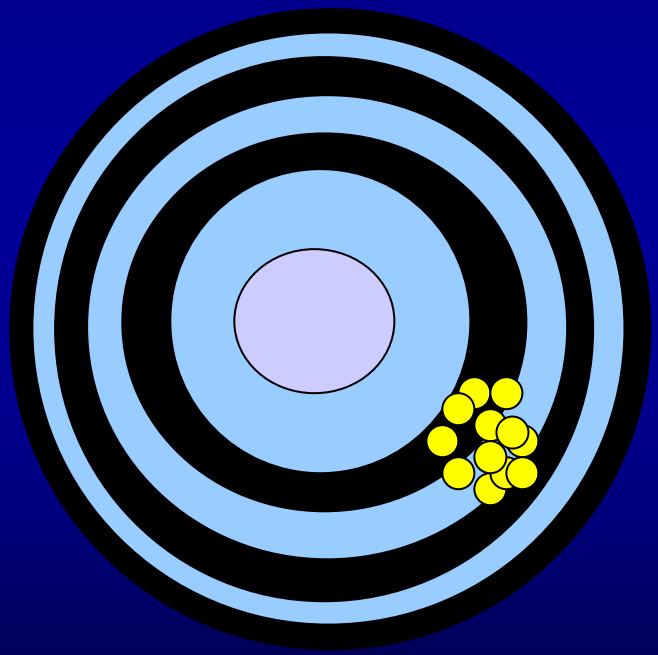


Accuracy and Precision

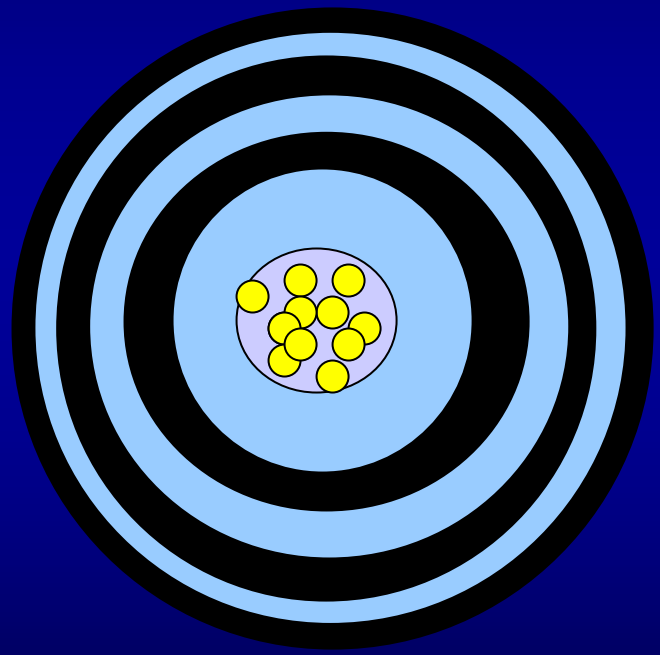
- The degree of fluctuation in the measurements is indicative of the “precision” of the assay.
- The closeness of measurements to the true value is indicative of the “accuracy” of the assay.
- Quality Control is used to monitor both the precision and the accuracy of the assay in order to provide reliable results.

Precision and Accuracy

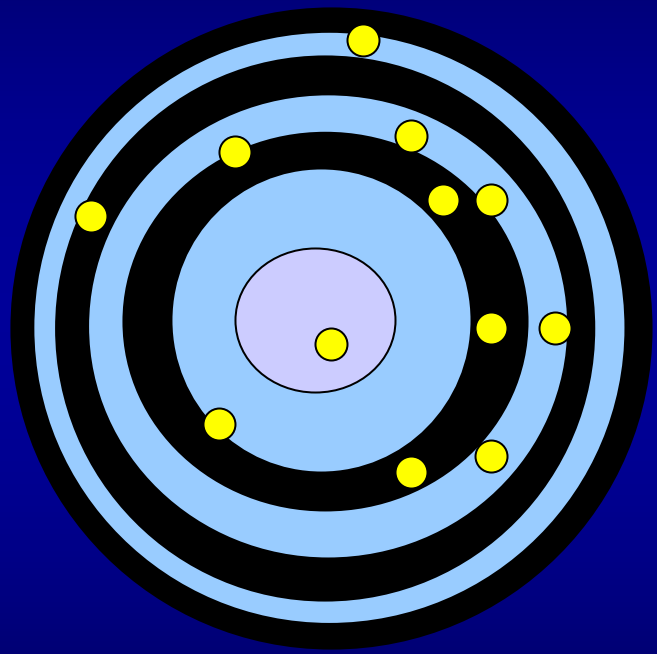
- Precise and inaccurate



- Precise and accurate



Imprecise and inaccurate



Measures of Dispersion or Variability

- There are several terms that describe the dispersion or variability of the data around the mean:

  Range

  Variance

  Standard Deviation

  Coefficient of Variation

Range

- Range refers to the difference or spread between the highest and lowest observations.
- It is the simplest measure of dispersion.
- It makes no assumption about the shape of the distribution or the central tendency of the data.

Calculation of Variance (S^2)

$$S^2 = \frac{\sum (X_1 - \bar{X})^2}{N-1} = mg^2/dl^2$$

Calculation of Variance

- Variance is a measure of variability about the mean.
- It is calculated as the average squared deviation from the mean.
  - the sum of the deviations from the mean, squared, divided by the number of observations (corrected for degrees of freedom)

Degrees of Freedom

- Represents the number of independent data points that are contained in a data set.

- The mean is calculated first, so the variance calculation has lost one degree of freedom (n-1)

Calculation of Standard Deviation

$$S = \sqrt{\frac{(x_1 - \bar{x})^2}{N-1}} = \text{mg/dl}$$

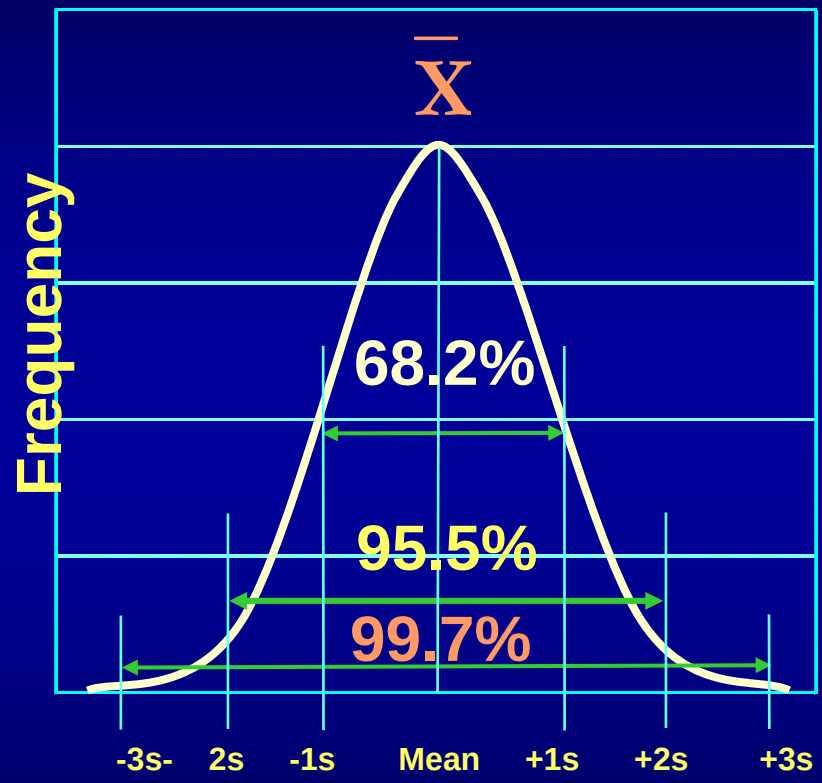
$$\sqrt{\text{variance}}$$

Calculation of Standard Deviation

- The standard deviation (SD) is the square root of the variance
  - it is the square root of the average squared deviation from the mean
- SD is commonly used (rather than the variance) since it has the same units as the mean and the original observations
- SD is the principle calculation used in the laboratory to measure dispersion of a group of values around a mean

Standard Deviation and Probability

- For a set of data with a normal distribution, a value will fall within a range of:
  - +/- 1 SD 68.2% of the time
  - +/- 2 SD 95.5% of the time
  - +/- 3 SD 99.7% of the time



Standard Deviation and Probability

- In general, laboratories use the +/- 2 SD criteria for the limits of the acceptable range for a test
- When the QC measurement falls within that range, there is 95.5% confidence that the measurement is correct
- Only 4.5% of the time will a value fall outside of that range due to chance; more likely it will be due to error

Calculation of Coefficient of Variation

- The coefficient of variation (CV) is the standard deviation (SD) expressed as a percentage of the mean
- Ideally should be less than 5%

$$CV = \frac{SD}{mean} \times 100$$

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Monitoring QC Data

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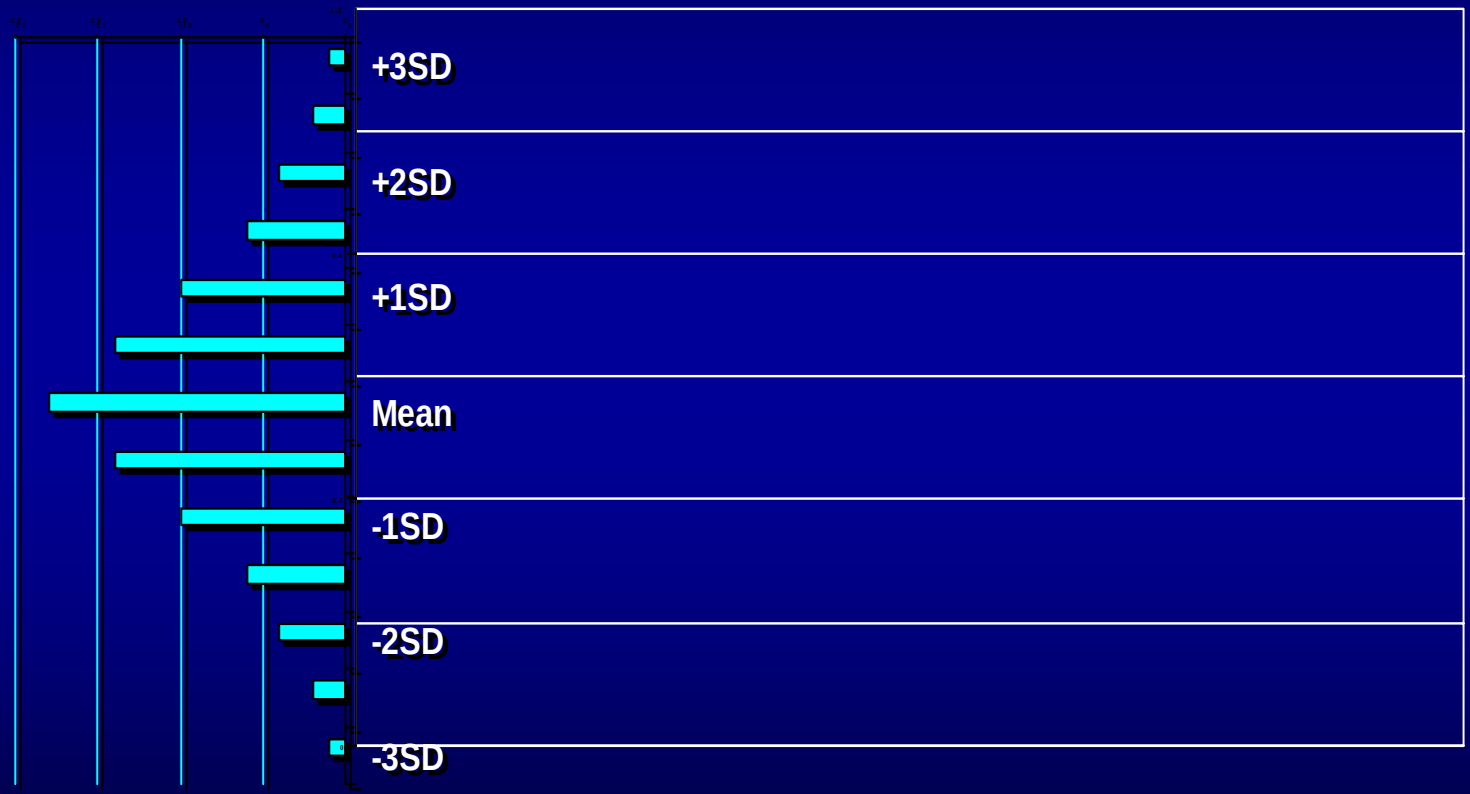
Monitoring QC Data

- Use Levey-Jennings chart
- Plot control values each run, make decision regarding acceptability of run
- Monitor over time to evaluate the precision and accuracy of repeated measurements
- Review charts at defined intervals, take necessary action, and document

Levey-Jennings Chart

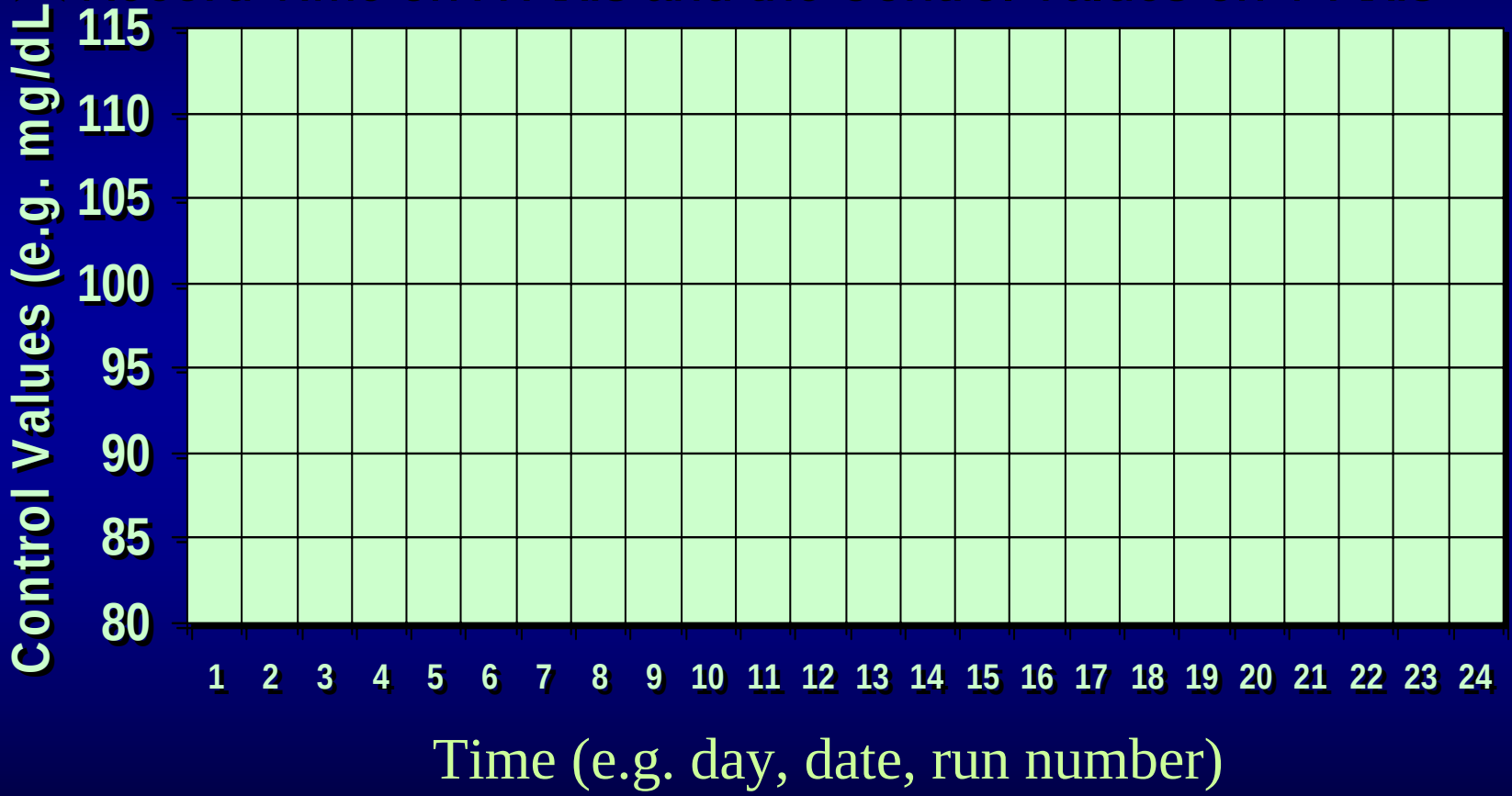
- A graphical method for displaying control results and evaluating whether a procedure is in-control or out-of-control
- Control values are plotted versus time
- Lines are drawn from point to point to accent any trends, shifts, or random excursions

Levey-Jennings Chart



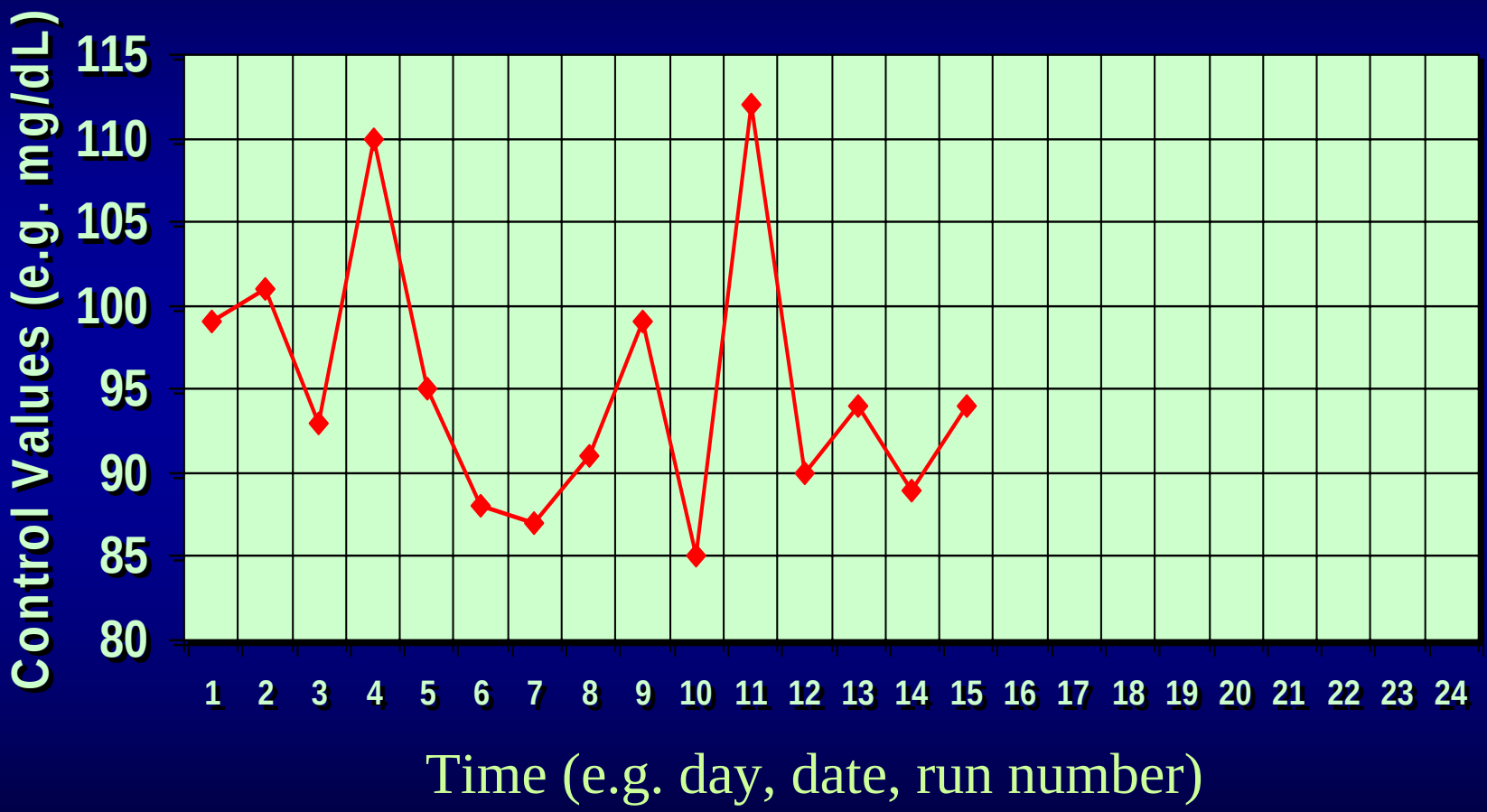
Levey-Jennings Chart -

Record Time on X-Axis and the Control Values on Y-Axis



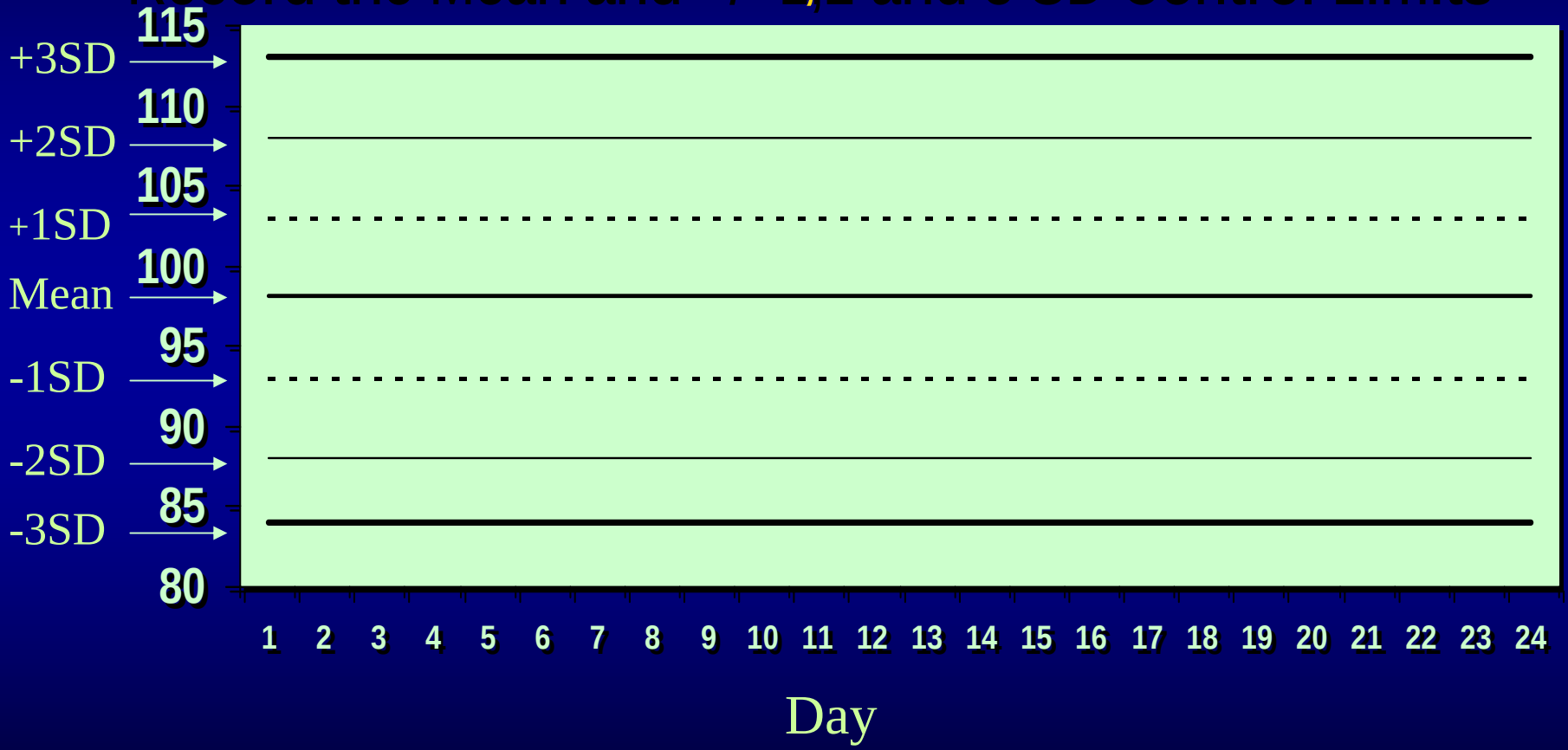
Levey-Jennings Chart -

Plot Control Values for Each Run

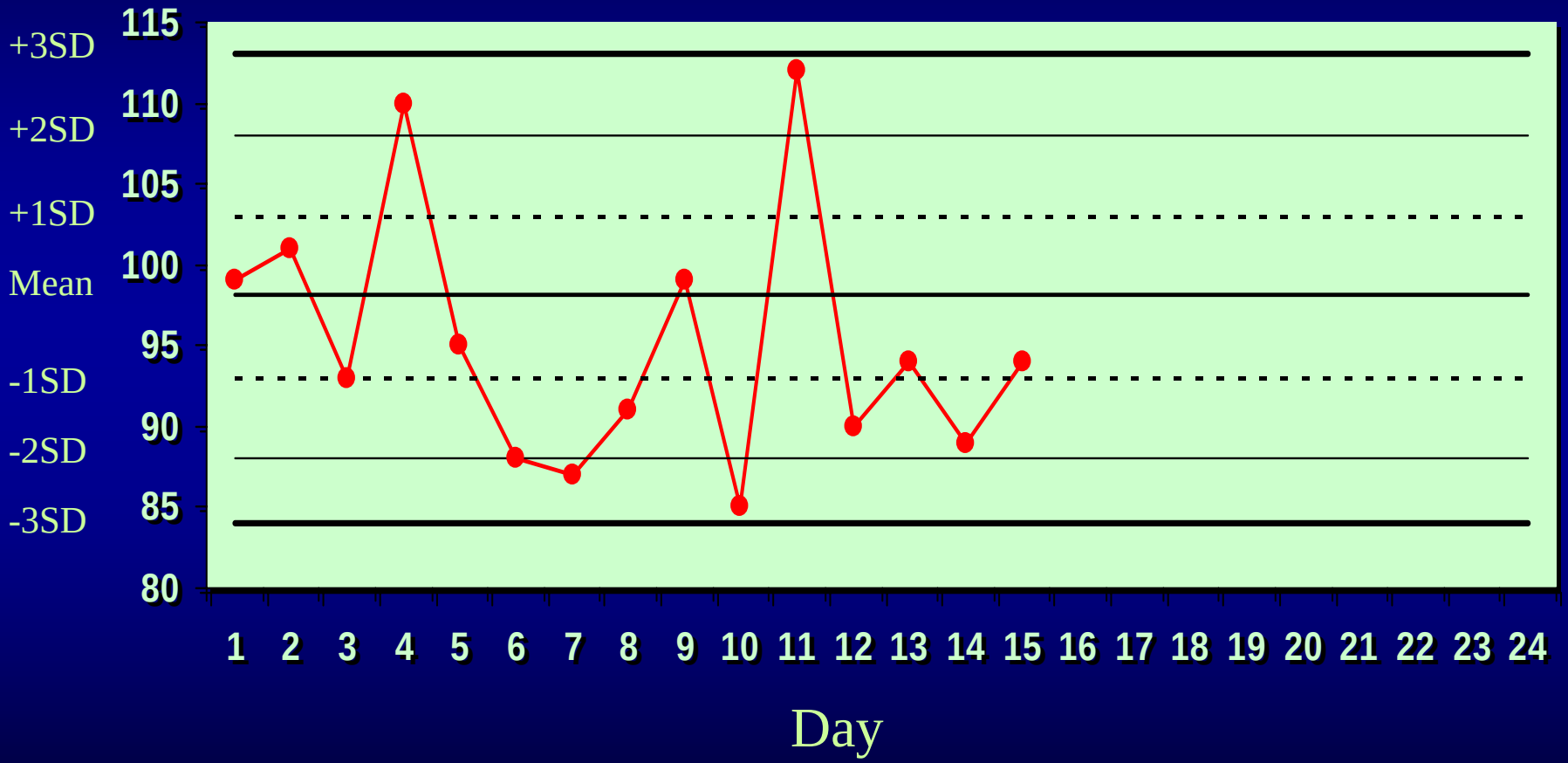


Levey-Jennings Chart

Calculate the Mean and Standard Deviation; Record the Mean and +/- 1,2 and 3 SD Control Limits



Levey-Jennings Chart -
Record and Evaluate the Control Values



Findings Over Time

- Ideally should have control values clustered about the mean (+/-2 SD) with little variation in the upward or downward direction
- Imprecision = large amount of scatter about the mean. Usually caused by errors in technique
- Inaccuracy = may see as a trend or a shift, usually caused by change in the testing process
- Random error = no pattern. Usually poor technique, malfunctioning equipment

Statistical Quality Control Exercise

- Hypothetical control values (2 levels of control)
- Calculation of mean
- Calculation of standard deviation
- Creation of a Levey-Jennings chart

When does the Control Value Indicate a Problem?

- Consider using Westgard Control Rules
- Uses premise that 95.5% of control values should fall within ±2SD
- Commonly applied when two levels of control are used
- Use in a sequential fashion

Westgard Rules

- “Multirule Quality Control”
- Uses a combination of decision criteria or control rules
- Allows determination of whether an analytical run is “in-control” or “out-of-control”

Westgard Rules

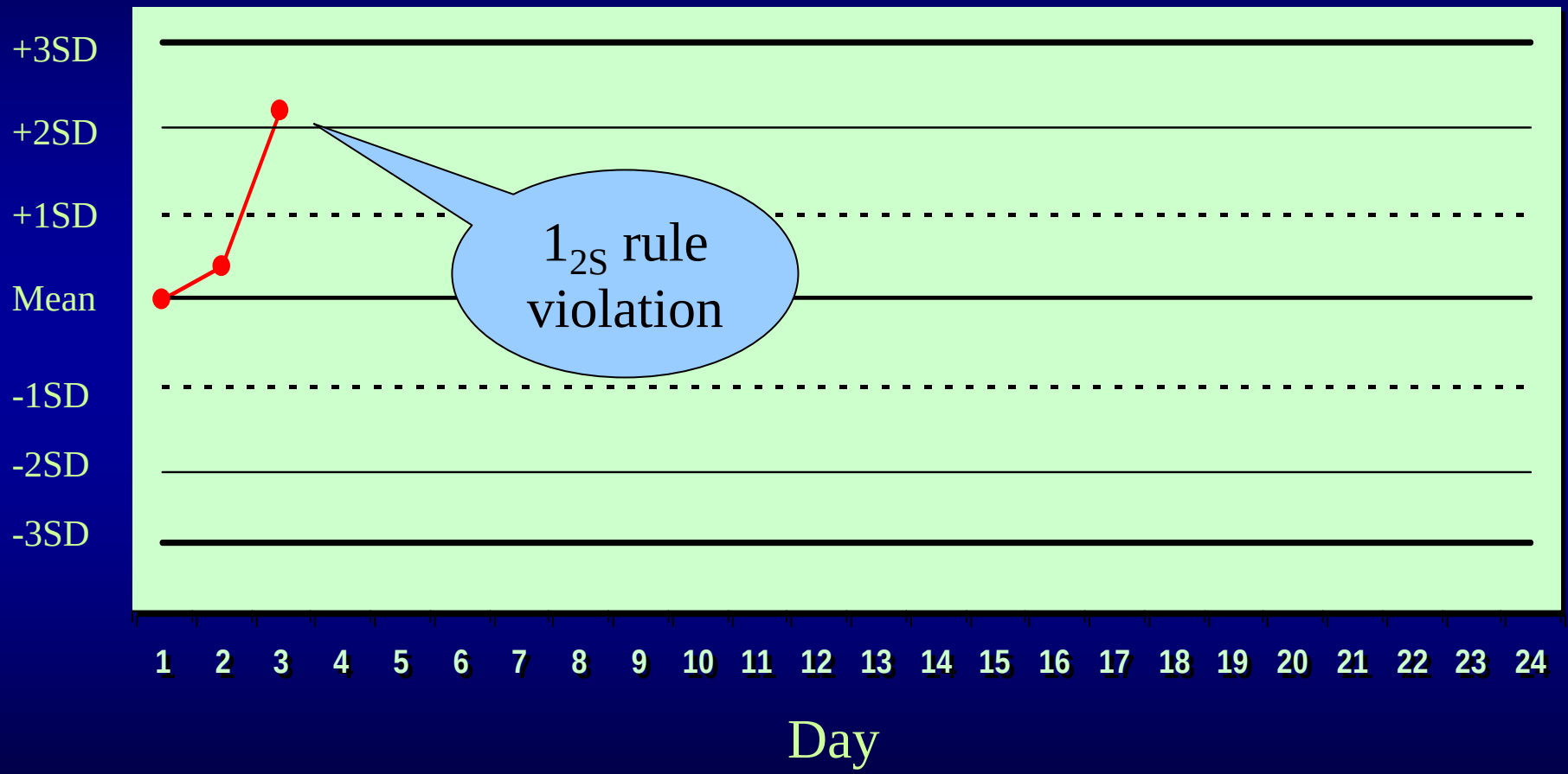
(Generally used where 2 levels of control material are analyzed per run)

- 1_{2S} rule
- 1_{3S} rule
- 2_{2S} rule
- R_{4S} rule
- 4_{1S} rule
- 10_{X} rule

Westgard – 1_{2S} Rule

- "warning rule"
- One of two control results falls outside ±2SD
- Alerts tech to possible problems
- Not cause for rejecting a run
- Must then evaluate the 1_{3S} rule

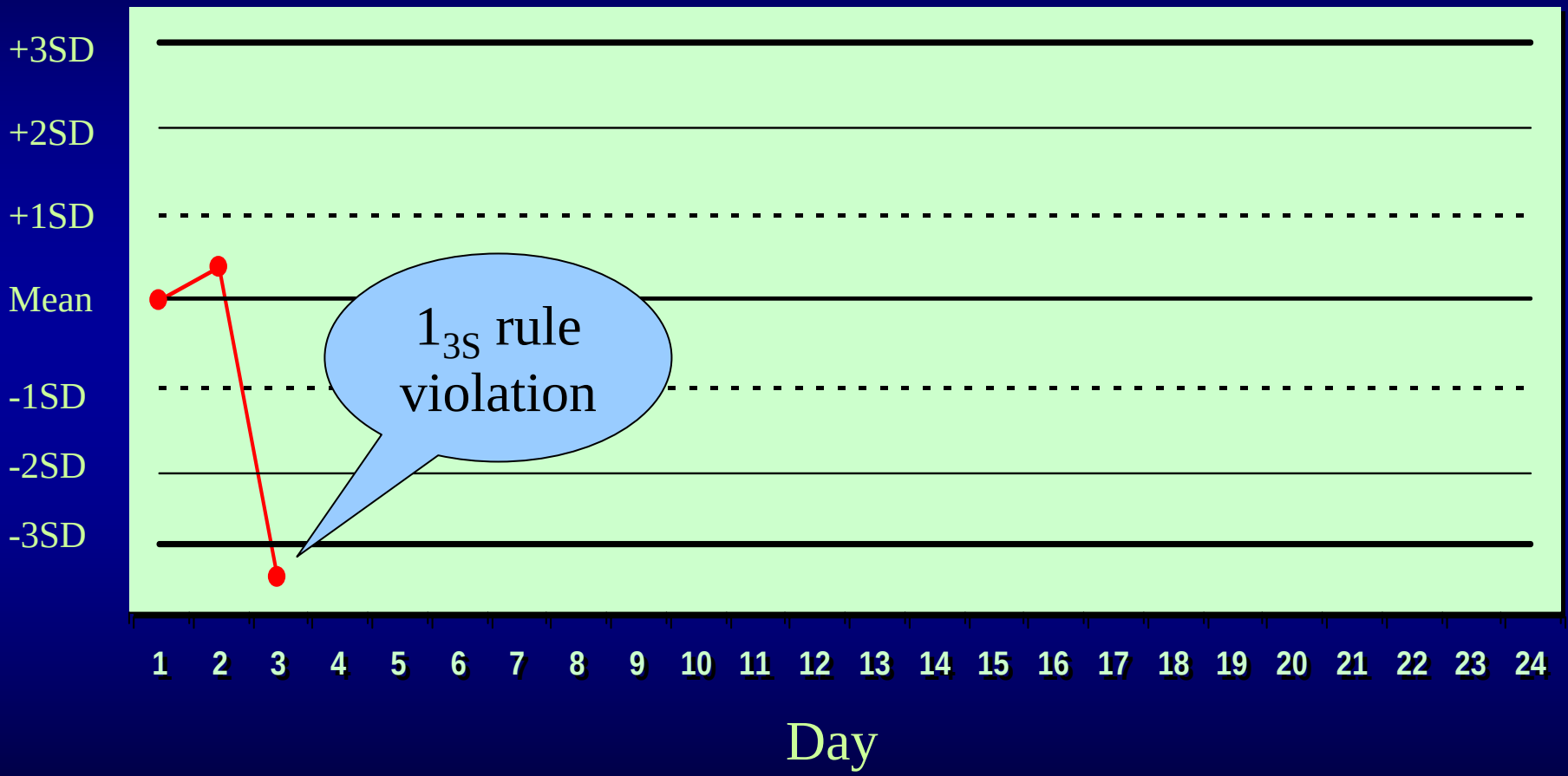
1_{2S} Rule = A warning to trigger careful inspection of the control data



Westgard – 1_{3S} Rule

- If either of the two control results falls outside of ±3SD, rule is violated
- Run must be rejected
- If 1_{3S} not violated, check 2_{2S}

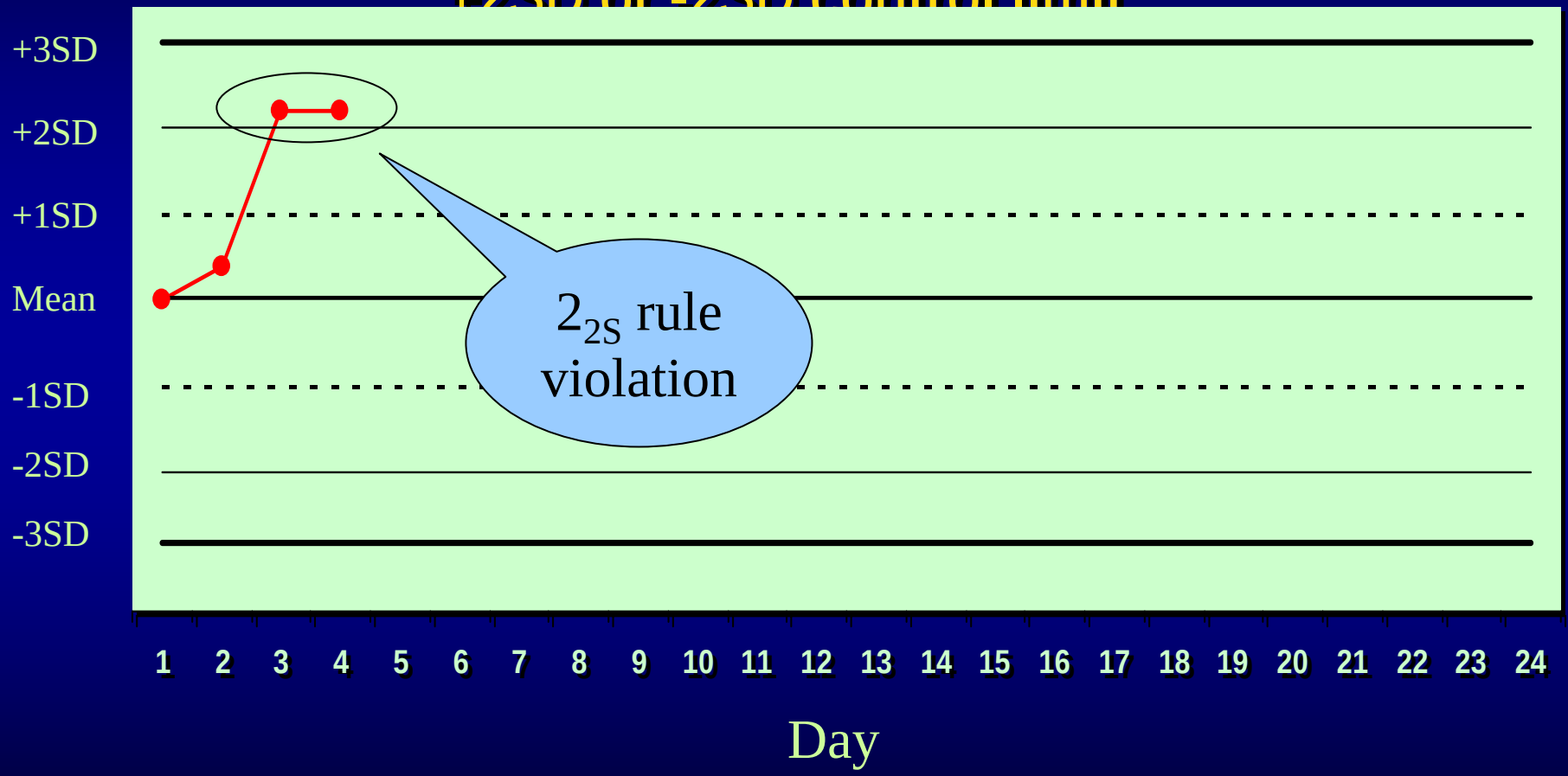
1_{3S} Rule = Reject the run when a single control measurement exceeds the +3SD or -3SD control limit



Westgard – 2_{2S} Rule

- 2 consecutive control values for the same level fall outside of ±2SD in the same direction, or
- Both controls in the same run exceed ±2SD
- Patient results cannot be reported
- Requires corrective action

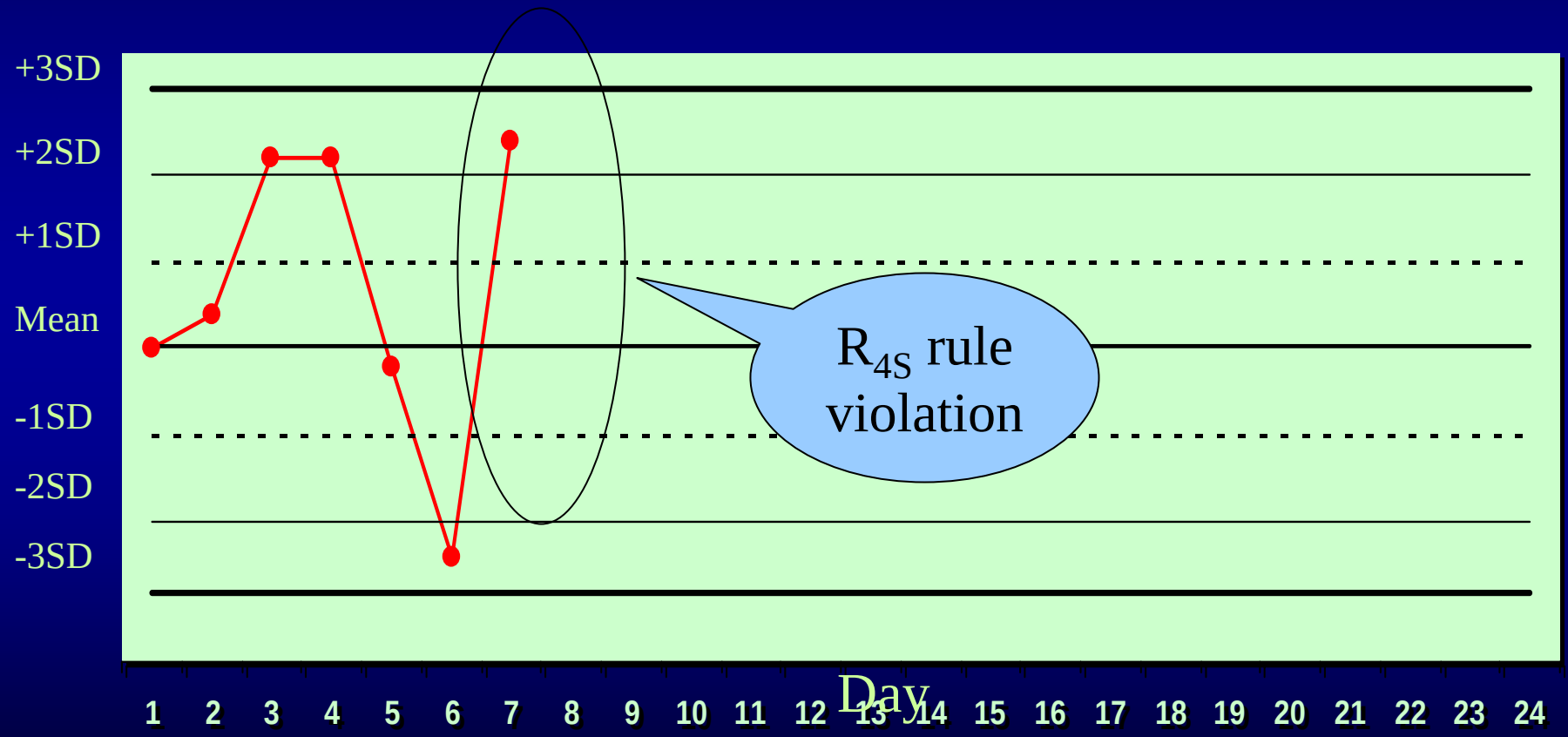
2_{2S} Rule = Reject the run when 2 consecutive control measurements exceed the same +2SD or -2SD control limit



Westgard – R_{4S} Rule

- One control exceeds the mean by –2SD, and the other control exceeds the mean by +2SD
- The range between the two results will therefore exceed 4 SD
- Random error has occurred, test run must be rejected

R_{4S} Rule = Reject the run when 1 control measurement exceed the +2SD and the other exceeds the -2SD control limit



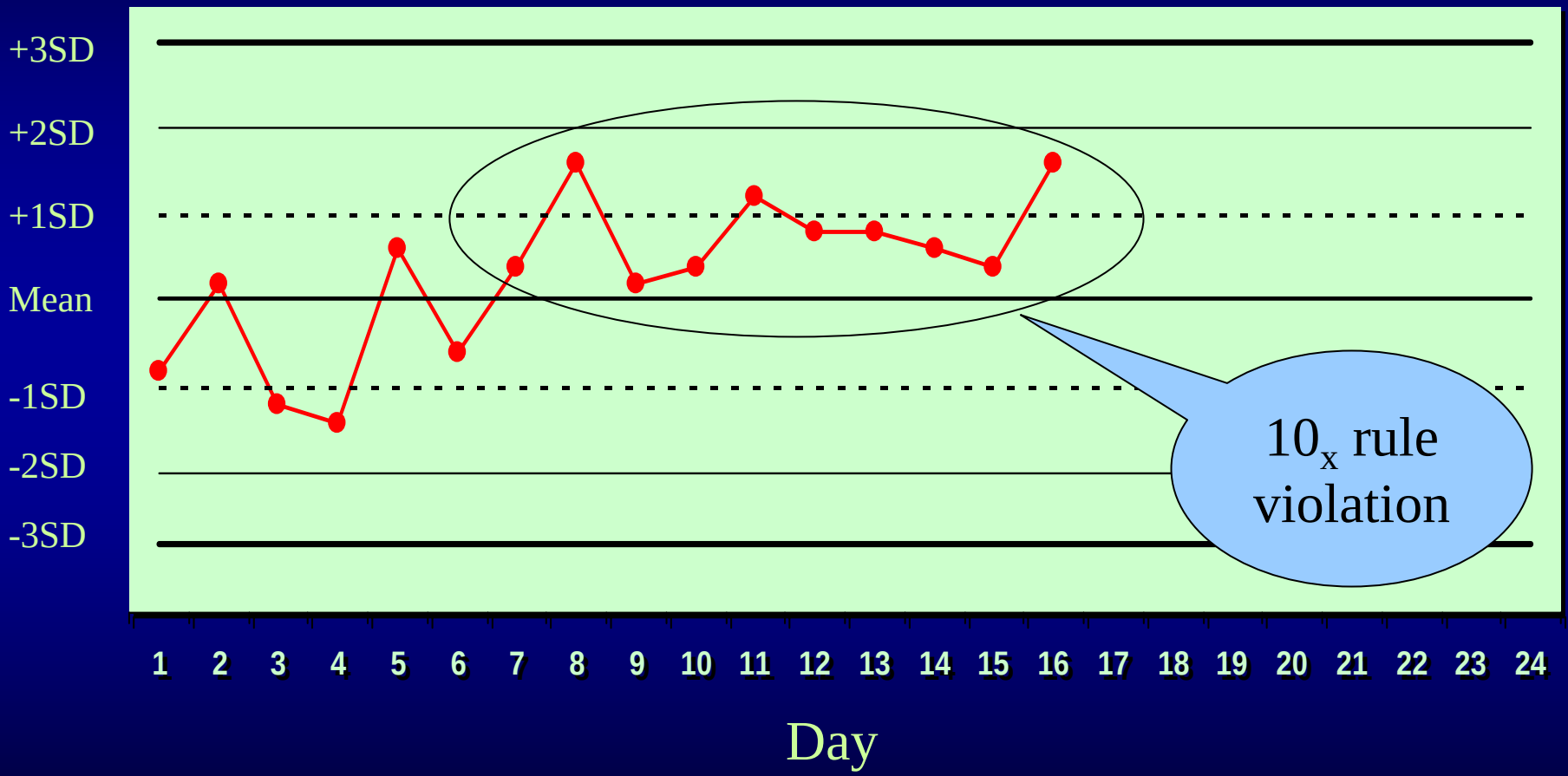
Westgard – 4_{1S} Rule

- Requires control data from previous runs
- Four consecutive QC results for one level of control are outside ±1SD, or
- Both levels of control have consecutive results that are outside ±1SD

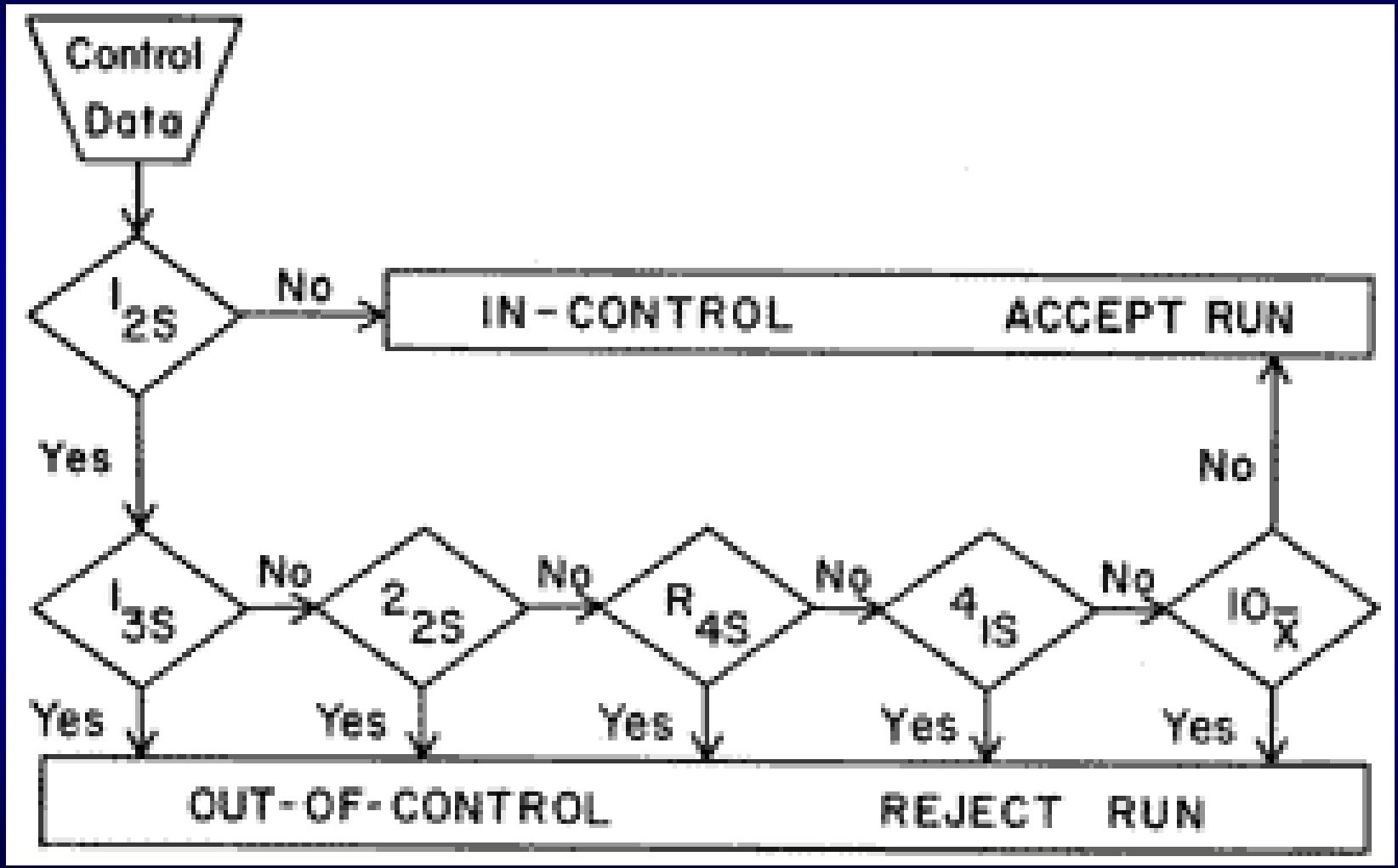
Westgard – 10_X Rule

- Requires control data from previous runs
- Ten consecutive QC results for one level of control are on one side of the mean, or
- Both levels of control have five consecutive results that are on the same side of the mean

10_x Rule = Reject the run when 10 consecutive control measurements fall on one side of the mean



Westgard Multirule QC



When a rule is violated

- Warning rule = use other rules to inspect the control points
- Rejection rule = “out of control”
  - Stop testing
  - Identify and correct problem
  - Repeat testing on patient samples and controls
  - Do not report patient results until problem is solved and controls indicate proper performance

Solving “out-of-control” problems

- Policies and procedures for remedial action
- Troubleshooting
- Alternatives to run rejection

Summary

- Why QC program?
  - Validates test accuracy and reliability

Summary: How to implement a QC program?

- Establish written policies and procedures
- Assign responsibility for monitoring and reviewing
- Train staff
- Obtain control materials
- Collect data
- Set target values (mean, SD)
- Establish Levey-Jennings charts
- Routinely plot control data
- Establish and implement troubleshooting and corrective action protocols
- Establish and maintain system for documentation